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Function of Rhodopsin in Temperature Discrimination in *Drosophila*

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Many animals, including the fruit fly, are sensitive to small differences in ambient temperature. The ability of *Drosophila* larvae to choose their ideal temperature (18°C) over other comfortable temperatures (19° to 24°C) depends on a thermosensory signaling pathway that includes a heterotrimeric guanine nucleotide–binding protein (G protein), a phospholipase C, and the transient receptor potential TRPA1 channel. We report that mutation of the gene (*ninaE*) encoding a classical G protein–coupled receptor (GPCR), *Drosophila* rhodopsin, eliminates thermotactic discrimination in the comfortable temperature range. This role for rhodopsin in thermotaxis toward 18°C was light-independent. Introduction of mouse melanopsin restored normal thermotactic behavior in *ninaE* mutant larvae. We propose that rhodopsins represent a class of evolutionarily conserved GPCRs that are required for initiating thermosensory signaling cascades.

Temperature sensation in animals is mediated largely by direct activation of transient receptor potential (TRP) ion channels (*1*–*3*). An exception is a TRP channel in *Drosophila* larvae that functions indirectly in the selection of their optimal temperature (18°C) over other comfortable temperatures (19° to 24°C) and does so through a signaling cascade that includes a heterotrimeric guanine nucleotide–binding protein (G protein) G_q, phospholipase C (PLC), and the TRPA1 channel (*4*). A thermosensory signaling cascade is also required in *Caenorhabditis elegans*, which includes guanylate cyclases and a guanosine 3′,5′-monophosphate (cGMP)–gated channel (*5*–*7*). Thermosensory signaling cascades may contribute to amplification of small temperature differences and to adaptation to temperatures that are less than optimal, but still permissive for survival (*4*, *8*).

G protein–coupled receptors (GPCRs) are candidates to initiate thermosensory cascades because they couple to pathways that include G_q, PLC, and TRP channels, as well as to cascades that engage guanylate cyclases and cGMP-gated channels. However, there are up to 200 hundred GPCRs encoded in flies (*9*) and over one thousand in worms (*10*), and there is no precedent for a GPCR that functions in thermosensation.

We wondered whether the canonical GPCR (rhodopsin) might be required for thermosensation, even though it is thought to function exclusively in light sensation. The basis for this proposal is that the same G_q (Gα49B) and PLC [No Receptor Potential A (NORPA)] that function in light sensation and link rhodopsin to activation of TRP channels are required for larvae to move preferentially toward the 18°C region when the alternative zone is held at another temperature in the 19° to 24°C range (*4*). If this behavior requires rhodopsin, it would be a light-independent function, because thermotaxis takes place effectively in the dark (*4*).

To test temperature selection, we placed larvae on a plate between two temperature zones, one of which was kept at 18°C and the other at an alternative temperature (*11*) (fig. S1A). After 10 min, we counted the larvae in each zone and calculated the preference index (PI) (fig. S1A). A lack of temperature bias results in a PI of 0, whereas a complete preference for 18°C or the alternative temperature results in a PI of 1.0 or –1.0, respectively. Wild-type larvae select 18°C over any other temperature, including other temperatures in their comfortable range (20° to 24°C) (Fig. 1A).

To address whether the major opsin (Rh1) encoded by the *ninaE* gene was required for thermotaxis in their comfortable temperature range, we tested flies with a deletion that removed the *ninaE* coding region ($ninaE^{I17}$). The ability to distinguish 18° from 24°C was impaired in $ninaE^{I17}$ larvae (Fig. 1, A and B) and in animals containing the $ninaE^{I17}$ mutation in trans with another deletion (Df) that removed *ninaE* on the homologous chromosome (Fig. 1A). This phenotype was indistinguishable from the thermotaxis deficits resulting from mutations disrupting PLC ($norpA^{P24}$) or the TRPA1 channel ($trpA1^{1}$) (*4*). Flies with any of five of six additional *ninaE* alleles showed deficits in discrimination between 18° and 24°C (Fig. 1B and fig. S1B), but not between 18°C and cooler or very warm temperatures (fig. S2, A and B). Larvae with one missense allele, $ninaE^{P332}$, strongly preferred 18°C over 24°C (Fig. 1B), although the bias for 18°C was eliminated

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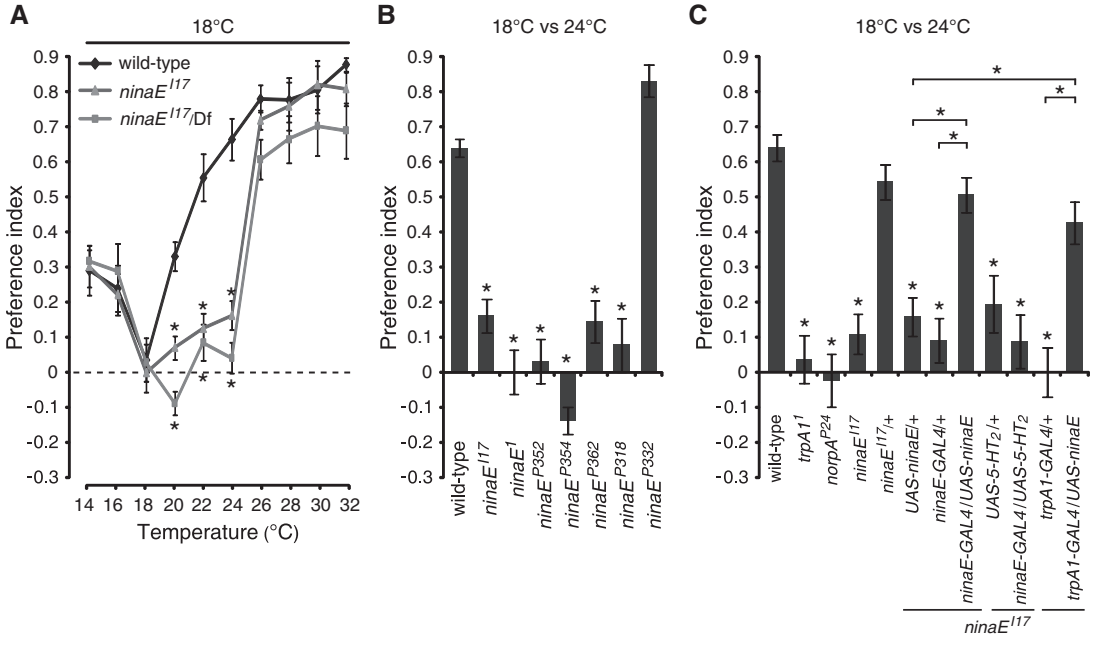

Fig. 1. Requirement for *ninaE* for larval thermotaxis. (**A**) Temperature preferences by using the binary choice assay. Larvae were given a choice between 18°C (top) and other temperatures (14° to 32°C) (bottom). Preferences for 18°C or the alternative temperature result in positive or negative PIs, respectively. (**B**) Assays of preference for 18° versus 24°C with multiple *ninaE* alleles. (**C**) Assays of preference for 18° versus 24°C with indicated genotypes. Error bars represent SEMs. Unless indicated otherwise, differences were relative to wild-type [*$P < 0.05$; (A) Tukey's analysis of variance (ANOVA); (B and C) Dunnett's ANOVA]. See tables S2 to S4 for statistics.

when the alternative temperature was either 20° or 22°C (fig. S2C).

To confirm that the thermotaxis defect was due to mutation of *ninaE*, we tested for rescue of the phenotype with a wild-type transgene, using the *GAL4-UAS* system (*12*). This approach employs the yeast GAL4 transcription factor that binds to the *upstream activation sequence* (*UAS*) to promote transcription. Only $ninaE^{I17}$ larvae containing both the *ninaE-GAL4* and *UAS-ninaE* transgenes effectively chose 18°C over 24°C (Fig. 1C). Another GPCR (serotonin receptor; *UAS-5-HT*$_2$), which is most similar to mammalian G_q-coupled serotonin receptors (*13*, *14*), does not rescue the $ninaE^{I17}$ deficit (Fig. 1C). Similar to the $norpA^{P24}$ and $trpA1^{1}$ phenotypes, loss of *ninaE* impaired discrimination between 18°C and other temperatures in the comfortable range, 20° or 22°C, but not selection of 18°C over cooler (14° or 16°C) or warmer temperatures (26° to 32°C) (Fig. 1A).

In *Drosophila*, the vitamin A–derived chromophore stably binds to the opsin and is required for Rh1 to exit the endoplasmic reticulum (*15*). Wild-type larvae grown on food depleted of vitamin A, or mutant larvae (*santa maria*^{1}) missing a scavenger receptor required for chromophore generation (*16*), showed impaired temperature discrimination in the 18°C to 24°C range (Fig. 2, A and B, and fig. S2D). The defect in *santa maria*^{1} was reversed by adding all *trans*-retinal to the food (R+) (Fig. 2B).

To address whether Rh1 might function in the same cells as other components involved in 18° to 24°C thermotaxis, we expressed *UAS-RNAi* transgenes under the transcriptional control of the *ninaE-GAL4* or the *trpA1-GAL4*. Expression of *Gα49B*, *norpA*, or *trpA1* RNA interference (RNAi) transgenes using the *ninaE-GAL4* reduced the biases toward 18°C over 22° or 23°C (Fig. 3A). Similarly, the preference for 18°C was diminished in larvae expressing the *ninaE* RNAi under control of the *trpA1-GAL4* (Fig. 3B and fig. S3). Expression of *UAS-ninaE*$^{+}$ under control of the *trpA1-GAL4* restored 18° versus 24°C temperature discrimination in $ninaE^{I17}$ larvae (Fig. 1C).

Because rhodopsin is a light sensor, we tested whether thermotactic behavior is altered by light.

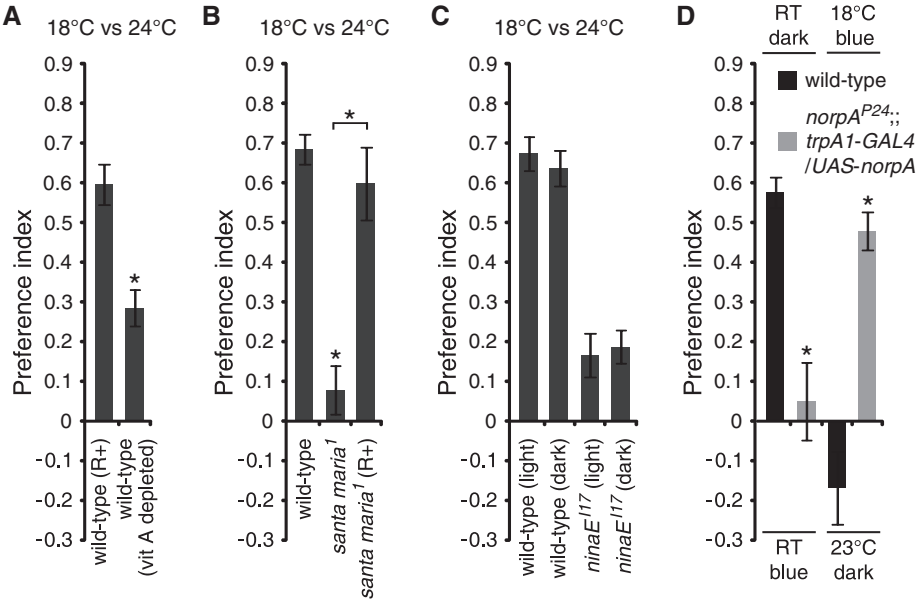

Fig. 2. Effects of chromophore and light on temperature selection. (**A**) Wild-type larvae were reared on retinal-deficient food (vit A depleted) or on the same food supplemented with all-*trans*-retinal (R+) and allowed to select 18° or 24°C. (**B**) Wild-type larvae were reared on instant fly food. *santa maria*^{1} larvae were reared on instant food or on instant food supplemented with all-*trans*-retinal (R+). (**C**) Assays of selection of 18° versus 24°C under ambient light (~0.035 mW/cm^2) or in the dark. (**D**) Wild-type larvae and mutants blind to moderate light ($norpA^{P24}$;;*trpA1-GAL4/UAS-norpA*) were placed on plates maintained uniformly at room temperature (RT; ~20°C) or on plates with two zones (18° and 23°C). The zones were kept in the dark or exposed to blue light (6.75 mW/cm^2). Error bars represent SEMs. Unless indicated otherwise, asterisk indicates significant differences relative to wild type [$P < 0.05$; (A, C, D), *t* test; (B), Tukey's ANOVA]. See tables S5 to S8 for statistics.

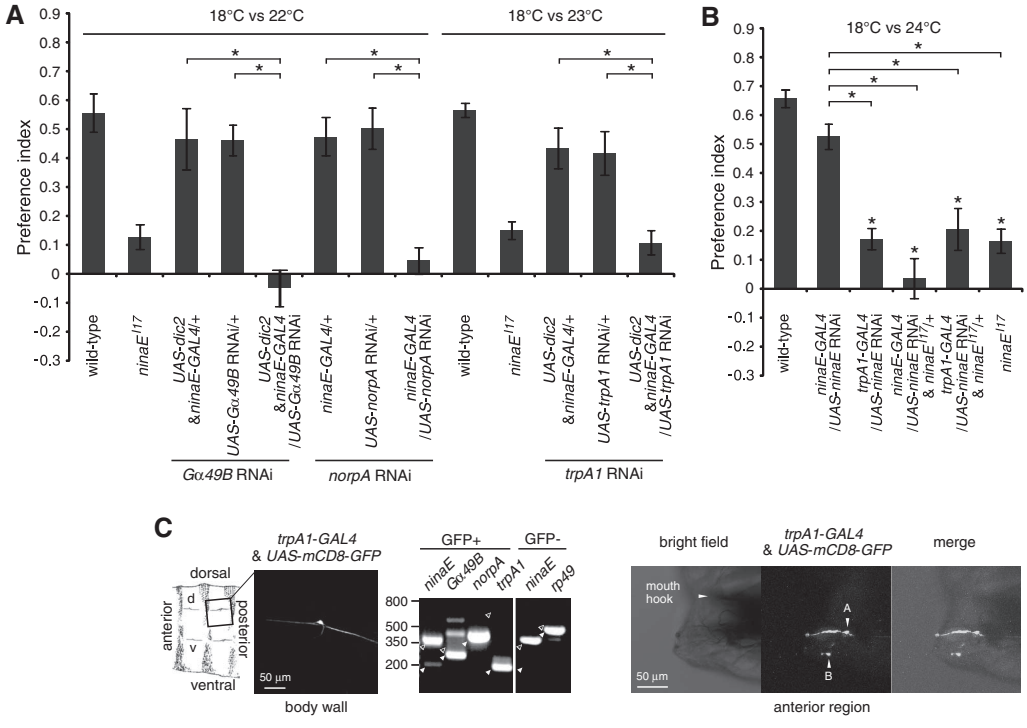

Fig. 3. Requirements for signaling components in the same cells. (**A**) Thermotactic behavior in larvae with *UAS*-RNAi transgenes expressed under control of the *ninaE-GAL4*. (**B**) Temperature selection after expression of *UAS-ninaE* RNAi under control of the *ninaE-GAL4* or the *trpA1-GAL4*. (**C**) RT-PCR analysis after manual dissection of GFP-positive neurons from the body wall or the anterior region of the larvae (*trpA1-GAL4* and *UAS-mCD8-GFP*), or after dissection of GFP-negative cells, which were close to v (vbd) or d (dbd). The reporter marked two morphologically similar neurons (dbd and vbd) in each body segment and two neurons near the anterior tip (A and B). The PCR primers spanned introns to distinguish products generated from RNA (white triangles) and genomic DNA (hollow triangles). Error bars represent SEMs. Unless indicated otherwise, asterisk indicates significant differences from wild-type [$P < 0.05$; (A), Tukey's ANOVA; (B), Dunnett's ANOVA]. See tables S9 and S10 for statistics.

Wild-type larvae chose 18° over 24°C equally well in the light or dark (Fig. 2C). Moreover, *ninaE*^{I17} displayed similar thermotactic impairments in the presence or absence of light (Fig. 2C). Thus, selection of 18° over 24°C was light-independent.

We also characterized larvae that were unresponsive to light. Wild-type early third instar larvae avoid white or blue, but not orange, light (Fig. 2D and fig. S4) (*17*). For larvae given a choice between 18° and 23°C, the aversion to light overcame the preference for 18°C (Fig. 2D and fig. S4B). Bolwig's organs, which consist of larval photoreceptor cells that function in the avoidance of moderate light intensities, do not express the *trpA1-GAL4* (fig. S5). *norpA*^{P24} animals are not negatively phototactic, and expression of *UAS-norpA*, under the control the *trpA1-GAL4* does not restore negative phototaxis (Fig. 2D and fig. S4B). These larvae discriminated temperatures in the 18° to 23°C range, and this behavior was not affected by light.

The *ninaE* gene appeared to be expressed at an exceptionally low level, because we were unable to detect a signal in larvae with Rh1 antibodies or using the *ninaE-GAL4* to drive *UAS-GFP*. Low amounts of Rh1 might prevent efficient light activation of Rh1 in thermosensory neurons, which might impair thermotactic discrimination. To provide additional evidence that *ninaE* was co-expressed with *trpA1*, we dissected neurons from the body wall and the anterior region that expressed the *trpA1*-reporter (*trpA1-GAL4* and *UAS-mCD8-GFP*; mCD8 is the mouse CD8 receptor), and we performed reverse transcription polymerase chain reaction (RT-PCR). We detected *ninaE* RT-PCR products in 5 out of 15 green fluorescent protein (GFP)–positive neurons (3 out of 8 from the body wall; 2 out of 7, anterior region), but not in any dissected GFP negative neurons (0 of 11; $P < 0.05$, Fisher's exact test) (Fig. 3C).

Selection of 17.5° to 18°C over cooler temperatures occurs through avoidance that results from increased turning at slightly lower temperatures (*18*, *19*). To test whether the preference for 18° over slightly higher temperatures occurred through a similar mechanism, we tracked larvae. Wild-type larvae appeared to progress only a short distance into the 24°C area before they paused, stretched their heads (movie S1), and initiated their first turns (fig. S6A). However, *ninaE*^{I17}, *norpA*^{P24}, and *trpA1*^{1} mutant larvae did not appear to turn until they traversed far into the 24°C zone (fig. S6A).

To quantify turning behavior, we developed a simple assay. We demarcated the 24°C zone with 20 lines (Fig. 4A), released the larvae on the 18°C side near the 24°C interface, and tabulated the last line crossed before the larvae made their first turn. We only counted larvae that moved perpendicular to the lines (≤5° deviation). Wild-type larvae turned near line 3 (Fig. 4B). However, the mutant larvae traveled to near line 14 in the 24°C area before turning (Fig. 4B). The much greater distances traveled by the mutants before turning did not appear to be due to increased movement speeds, because all the larvae moved at similar rates (fig. S6B). In a reciprocal experiment, we placed larvae on the 24°C side and monitored animals that crossed perpendicular to the lines demarcating the 18°C zone. Wild-type larvae did not turn until line 10, and there were only small variations between wild-type and mutant animals (Fig. 4B).

We tested whether the higher rate of larval turning at 24°C was dependent on prior exposure to a lower temperature. Wild-type larvae placed on a plate uniformly held at a single temperature showed similar turning frequencies at all temperatures tested (18° to 24°C) (fig. S6C). We obtained similar results with the *ninaE*^{I17}, *norpA*^{P24}, and *trpA1*^{1} larvae (fig. S6C). Thus, turning at 24°C was dependent on prior exposure to 18°C.

Several results argue strongly against a developmental defect underlying the thermotaxis impairment in the comfortable range. First, although *ninaE*^{P332} larvae were impaired in selecting 18°C over 20° or 22°C, they were able to choose 18°C over 24°C (fig. S2C). Second, multiple *ninaE* missense mutations, including *ninaE*^{P332} and *ninaE*^{P318}, have no apparent effects on morphogenesis and are not associated with retinal degeneration (*20*), which suggests that these alleles do not affect development of the thermosensory neurons. Third, we found indistinguishable numbers and morphological appearances of GFP-positive cells in wild-type and *ninaE*^{I17} larvae that expressed *UAS-mCD8-GFP* under control of the *trpA1-GAL4* (table S1 and fig. S7A).

We took advantage of the slightly higher PI exhibited by *ninaE*^{P332} (18° versus 24°C) to test whether other genes required for thermotaxis functioned subsequent to *ninaE*. Introduction of the *Gα49B*^{1}, *norpA*^{P24} or *trpA1*^{1} mutations into the *ninaE*^{P332} background prevented 18°C selection over 24°C (fig. S7B). Another mutation that causes a higher-than-normal PI disrupts the rhodopsin phosphatase (*rdgC*^{306}) (*4*). The combination of *ninaE*^{I17} or *Gα49B*^{1} with *rdgC*^{306} eliminated the bias for 18° over 24°C (fig. S7B). These analyses indicate that G_q, PLC, and TRPA1 function in a pathway downstream of Rh1.

Drosophila encodes additional opsins (Rh2-6) (*15*). To determine whether other opsins could substitute for Rh1, we expressed Rh2-6 under control of the *ninaE* promoter in *ninaE*^{I17} flies and assayed 18° versus 24°C selection. With the

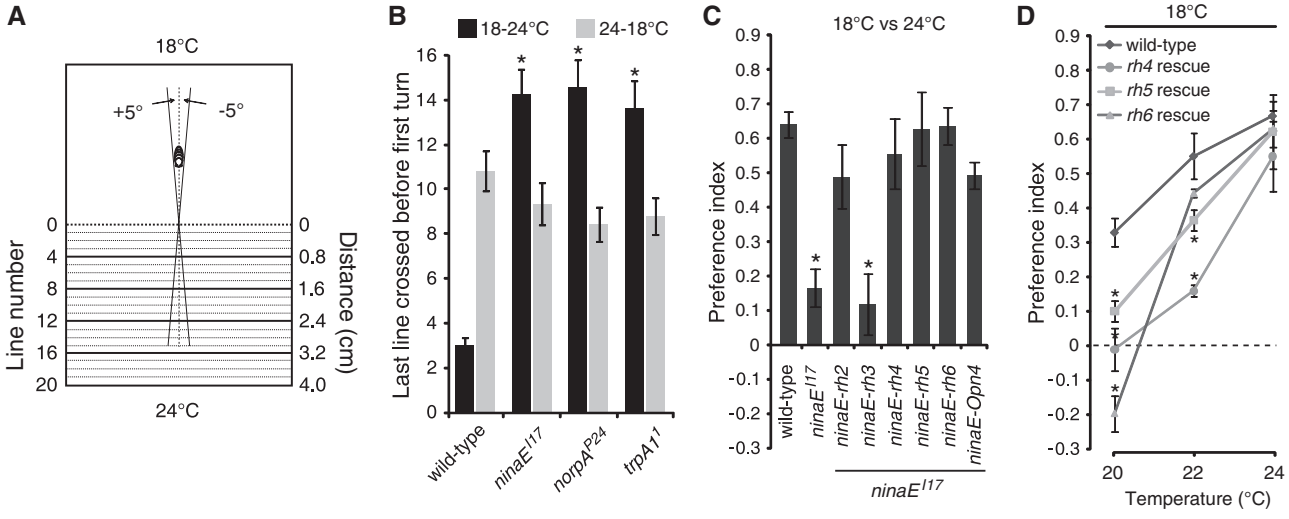

Fig. 4. Temperature-dependent turning and thermotactic behavior of larvae expressing other opsins in place of Rh1. (**A**) Set-up for quantitative analysis of turning behavior. To assay turning behavior from 18° to 24°C, the 24°C side was demarcated with 20 lines. We released larvae within the 18°C zone and tracked larvae that crossed the midline (line 0) and moved perpendicular to the lines (±5°). We tabulated the last line crossed before the larvae made the first turn. To assay turning from 24° to 18°C, we released larvae on the 24°C side. (**B**) Last line crossed before larvae made the first turn at 18° to 24°C and 24° to 18°C. (**C**) Rescue of *ninaE*^{I17} thermotactic defect by expression of other fly opsins or mouse *Opn4* under control of the *ninaE* promoter. (**D**) Thermal preferences with Rh4 and Rh6 in place of Rh1 (18°C versus the indicated temperature). Error bars indicate SEMs. Asterisks indicate significant differences from wild-type ($P < 0.05$; Dunnett's ANOVA test). See tables S11 to S13 for detailed statistics.

exception of Rh3, other opsins could replace Rh1 (Fig. 4C). However, the transgenic flies showed significant differences from wild type when given a choice between 18° and 20° to 22°C (Fig. 4D). Another GPCR coupled to G_q [5-hydroxytryptamine (5-HT_2)] did not function in place of Rh1 (Fig. 1C).

The mammalian opsin that is most similar to *Drosophila* Rh1 is melanopsin (OPN4) (*21*). Expression of *Opn4* under control of the *ninaE* promoter did not reverse the phototransduction defect in adult $ninaE^{I17}$ (fig. S8). However, *Opn4* enabled the $ninaE^{I17}$ larvae to distinguish between 18°C and 24°C (Fig. 4C).

The observations that Rh1 is required for thermosensory discrimination and that OPN4 could substitute for Rh1 suggest that Rh1 and related opsins might be intrinsic thermosensors. However, the intrinsic rate of thermal activation, which is ~1/min in fly photoreceptor cells (*22*), is far too low to account for the requirement for Rh1 for thermosensation. We suggest that an accessory factor might interact with Rh1 and accelerates its intrinsic thermal activity. Finally, because rhodopsin has dual roles, it is interesting to consider the question as to whether the archetypal role for rhodopsin was in light sensation or in thermosensation.

References and Notes

1. M. J. Caterina, *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **292**, R64 (2007).
2. M. Bandell, L. J. Macpherson, A. Patapoutian, *Curr. Opin. Neurobiol.* **17**, 490 (2007).
3. K. Venkatachalam, C. Montell, *Annu. Rev. Biochem.* **76**, 387 (2007).
4. Y. Kwon, H. S. Shim, X. Wang, C. Montell, *Nat. Neurosci.* **11**, 871 (2008).
5. C. M. Coburn, C. I. Bargmann, *Neuron* **17**, 695 (1996).
6. H. Komatsu, I. Mori, J. S. Rhee, N. Akaike, Y. Ohshima, *Neuron* **17**, 707 (1996).
7. H. Inada *et al.*, *Genetics* **172**, 2239 (2006).
8. D. Ramot, B. L. MacInnis, M. B. Goodman, *Nat. Neurosci.* **11**, 908 (2008).
9. T. Brody, A. Cravchik, *J. Cell Biol.* **150**, F83 (2000).
10. C. I. Bargmann, *Science* **282**, 2028 (1998).
11. Materials and methods are available as supporting material on *Science* Online.
12. A. H. Brand, N. Perrimon, *Development* **118**, 401 (1993).
13. J. F. Colas, J. M. Launay, O. Kellermann, P. Rosay, L. Maroteaux, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 5441 (1995).
14. M. Filip, M. Bader, *Pharmacol. Rep.* **61**, 761 (2009).
15. T. Wang, C. Montell, *Pflugers Arch.* **454**, 821 (2007).
16. T. Wang, Y. Jiao, C. Montell, *J. Cell Biol.* **177**, 305 (2007).
17. P. H. Strange, *J. Exp. Biol.* **38**, 237 (1961).
18. Y. Kwon, W. L. Shen, H. S. Shim, C. Montell, *J. Neurosci.* **30**, 10465 (2010).
19. L. Luo *et al.*, *J. Neurosci.* **30**, 4261 (2010).
20. J. P. Kumar, D. F. Ready, *Development* **121**, 4359 (1995).
21. I. Provencio, G. Jiang, W. J. De Grip, W. P. Hayes, M. D. Rollag, *Proc. Natl. Acad. Sci. U.S.A.* **95**, 340 (1998).
22. R. C. Hardie *et al.*, *Neuron* **36**, 689 (2002).

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Supporting Online Material

www.sciencemag.org/cgi/content/full/331/6022/1333/DC1
Materials and Methods
Figs. S1 to S8
Tables S1 to S17
References
Movie S1

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A Polarized Epithelium Organized by β- and α-Catenin Predates Cadherin and Metazoan Origins

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A fundamental characteristic of metazoans is the formation of a simple, polarized epithelium. In higher animals, the structural integrity and functional polarization of simple epithelia require a cell-cell adhesion complex that contains a classical cadherin, the Wnt-signaling protein β-catenin and the actin-binding protein α-catenin. We show that the non-metazoan *Dictyostelium discoideum* forms a polarized epithelium that is essential for multicellular development. Although *D. discoideum* lacks a cadherin homolog, we identify an α-catenin ortholog that binds a β-catenin–related protein. Both proteins are essential for formation of the epithelium, polarized protein secretion, and proper multicellular morphogenesis. Thus, the organizational principles of metazoan multicellularity may be more ancient than previously recognized, and the role of the catenins in cell polarity predates the evolution of Wnt signaling and classical cadherins.

A simple epithelium is the most basic tissue type in metazoans (multicellular animals). It is the first overt sign of cellular differentiation during embryogenesis and is important for the morphogenesis of many tissues and homeostasis in the adult (*1*). A simple epithelium comprises a cell monolayer surrounding a luminal space. The cells have a polarized organization of plasma membrane proteins, organelles, and cytoskeletal networks that together regulate the directional absorption and secretion of proteins and other solutes (*1*).

The structural integrity and functional polarity of epithelial tissues in higher animals require cell-cell adhesion mediated by classical cadherins (*2*). Adhesion provides a spatial cue that initiates cell polarization via recruitment of cadherin-associated cytosolic proteins (*3*), including the Wnt-signaling protein β-catenin (*4*) and the actin-binding protein α-catenin (*5*). Classical cadherins, which have extracellular cadherin repeats (*6*) and a conserved cytoplasmic domain that can bind β-catenin (*7*), are found in all multicellular animals, including sponges, but not in choanoflagellates (*8*–*10*), which suggests that classical cadherins are restricted to metazoans. However, the evolutionary history of the catenins is unknown, and thus how the cadherin-catenin complex evolved to mediate epithelial polarity in metazoans is unclear.

The non-metazoan social amoeba *Dictyostelium discoideum* undergoes multicellular morphogenesis in response to starvation: Single cells aggregate and undergo culmination to form a fruiting body, which comprises a rigid stalk that supports a collection of spores (Fig. 1A) (*11*). The mechanical rigidity of the stalk is due to the stalk tube, which contains cellulose and the extracellular matrix proteins EcmA/B (Fig. 1B) (*12*, *13*). Harwood and colleagues described a ring of cells surrounding the stalk tube at the tip of the culminant and speculated that these cells might contribute to stalk formation during culmination (*14*, *15*). However, the subcellular organization and function of tip cells have not been characterized.

We confirmed the earlier observation (*14*) that the tip consists of an organized monolayer of cells surrounding the stalk (Fig. 1, A and B, and movie S1). Additionally, we found that these cells have a distinctive polarized organization: Centrosomes and Golgi localized to a stalk side of nuclei (Fig. 1C), and the transmembrane protein cellulose synthase [encoded by the *dcsA* gene (*12*)] localized to the plasma membrane domain adjacent to the stalk tube (Fig. 1D). Thus, *D. discoideum* tip cells have a subcellular organization that is characteristic of a simple polarized epithelium (fig. S1), and we refer to these cells as the tip epithelium.

In metazoans, β-catenin and α-catenin are essential for the formation of polarized simple epithelia (*16*, *17*). A β-catenin–related protein called Aardvark has been identified in *D. discoideum* (fig. S2) (*9*, *14*). We identified a member of the α-catenin family in this organism, which we

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